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Transfection of chimeric anti-CD138 gene enhances natural killer cell activation and killing of multiple myeloma cells☆

Hua Jiang[a,1], *Wenhao Zhang*[a,1], *Peipei Shang*[a,1], *Hui Zhang*[a], *Weijun Fu*[a], *Fei Ye*[a], *Tianmei Zeng*[a], *Hejing Huang*[a], *Xueguang Zhang*[b], *Wanping Sun*[b], *Daniel Man-Yuen Sze*[c], *Qing Yi*[d], *Jian Hou*[a,*]

[a]*Department of Hematology, The Myeloma and Lymphoma Center, Changzheng Hospital, The Second Military Medical University, 415 Fengyang Rd, Shanghai 200003, China*

[b]*Institute of Biotechnology and Clinical Immunology, Research Laboratory of Jiangsu Province, Soochow University, Suzhou 215007, China*

[c]*Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong Special Administrative Region*

[d]*Department of Lymphoma/Myeloma, Division of Cancer Medicine, and Center for Cancer Immunology Research, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA*

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ABSTRACT

Reprogramming of NK cells with a chimeric antigen receptor (CAR) proved an effective strategy to increase NK cell reactivity and recognition specificity toward tumor cells. To enhance the cytotoxicity of NK cells against CD138-positive multiple myeloma (MM) cells, we generated genetically modified NK-92MI cells carrying a CAR that consists of an anti-CD138 single-chain variable fragment (scFv) fused to the CD3ζ chain as a signaling moiety. The genetic modification through a lentiviral vector did not affect the intrinsic cytolytic activity of NK-92MI toward human erythroleukemic cell line K562 cells or CD138-negative targets. However, these retargeted NK-92MI (NK-92MI-scFv) displayed markedly enhanced cytotoxicity against CD138-positive human MM cell lines (RPMI8226, U266 and NCI-H929) and primary MM cells at various effector-to-target ratios (E:T) as compared to the empty vector-transfected NK-92MI (NK-92MI-mock). In line with the enhanced cytotoxicity of NK-92MI-scFv, significant elevations in the secretion of granzyme B, interferon-γ and proportion of CD107a expression were also found in NK-92MI-scFv in response to CD138-positive targets compared with NK-92MI-mock. Most importantly, the enhancement in the cytotoxicity of NK-92MI-scFv did not attenuate with 10Gy-irradiation that sufficiently blocked cell proliferation. Moreover, the irradiated NK-92MI-scFv exerted definitely intensified anti-tumor activity toward CD138-positive MM cells than NK-92MI-mock in the xenograft NOD-SCID mouse model. This study provides the rationale and feasibility for adoptive

Abbreviations: α-MEM, alpha modification of Eagle's minimum essential medium; ATCC, American type culture collection; CAR, chimeric antigen receptor; GvM, graft-vs-myeloma effect; IMiDs, immunomodulatory drugs; KIRs, immunoglobulin-like receptors; MFI, mean fluorescence intensity; MM, multiple myeloma; PBMNC, peripheral blood mononuclear cell; PCL, plasma cell leukemia; RACE, rapid amplification of cDNA ends; scFv, single-chain variable fragment; SP, signal peptide; TCR, T-cell receptor.

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* *Corresponding author*. Tel.: +86 21 81885421; fax: +86 21 63583688.
E-mail address: houjian167@sohu.com (J. Hou).

[1] These authors contributed equally to this work and are considered co-first authors.

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immunotherapy with CD138-specific CAR-modified NK cells in CD138-positive plasmacytic malignancies, which potentially further improves remission quality and prolongs the remission duration of patients with MM after upfront chemotherapy.

1. Introduction

Multiple myeloma (MM) remains an incurable malignant plasma cell disorder. Despite the markedly improved outcome in recent years, the vast majority of patients with MM could not escape disease relapse or progression (Hart et al., 2012). Thus, novel strategies are urgently needed to further increase the response frequency and quality, and prolong the overall survival and disease free survival of patients with MM.

Human natural killer (NK) cells play an essential role in innate immune defense against malignant cells, potentiating it to be a satisfying effector cells for adoptive immunotherapy (Vivier et al., 2011). It has been demonstrated that NK cells mediate graft-vs-myeloma effect (GvM) in MM patients undergoing allogenic hematopoietic cell transplantation (Reynolds et al., 2001). A recent study indicated that NK cells were capable to kill clonogenic MM cells *in vitro* and *in vivo* (Swift et al., 2012). The striking efficacy of the upfront immunomodulatory drugs (IMiDs) in maintenance therapy of MM, have been identified to be closely related to its positive influence on NK cell function (McDaniel et al., 2012). All these results suggested that adoptive immunotherapy with NK cells provides a promising treatment modality for eradication or control of the residual MM cells, potentially complementing the first-line therapies.

However, adoption of primary allogeneic or autologous NK cells is largely limited by difficulties in *ex vivo* cell expansion as well as the variation in NK cell activity from different patients (Tonn et al., 2001), which made the established NK cell lines an attractive option as effector cells for immunotherapy. NK-92 is the only NK cell line to be tested in clinical trials for immunotherapy of malignancies, and its safety and expansion feasibility have been validated in phase I trial in renal cell cancer or melanoma (Arai et al., 2008). NK-92 cells lack almost all inhibitory killer cell immunoglobulin-like receptors (KIRs) except KIR2DL4, which inhibit NK cell activation by binding to HLA molecule on target cells (Tonn et al., 2001). The lack of KIRs on NK-92 cells may, at least in part, account for its marked anti-tumor activity against a broad spectrum of tumor targets (Morett et al., 2001). NK-92MI is an interleukin-2 (IL-2) independent derivative cell line of NK-92 by transfection of human IL-2 cDNA, with the same characteristics of activated NK cells as its parental NK-92 cells (Favors et al., 2012). Reprogramming of NK cells with a chimeric antigen receptor (CAR) proved an effective strategy to enhance their reactivity against the antigen-expressing tumor cells or overcome resistance (Boissel et al., 2009, 2012; Tassev et al., 2012).

CD138 (syndecan-1) is an integral membrane protein widely expressed on differentiated plasma cells, and has been taken as a primary diagnostic marker of MM (Lutz and Whiteman, 2009). It acts as a receptor for the extracellular matrix through its extracellular domain, mediating MM development and proliferation (Dhodapkar et al., 1998; Bataille et al., 2006). The high expression of CD138 on MM cells potentiates it to be a specific immunotherapeutic target for MM. To enhance the cytotoxicity of NK-92MI to CD138 expressing MM cells, we transfected NK-92MI cells with a lentiviral vector encoding a recombinant CAR termed scFv (4B3)-CD3ζ that is CD138-specific single-chain antibody fragments (scFv) genetically fused to the CD3ζ chain of the T-cell receptor (TCR) complex (another signaling molecule known to trigger cytotoxicity of NK cells) (André et al., 2004; Imai et al., 2005), via a flexible hinge region of CD8. Then we detected the expression of CAR on the transfected NK cells, and examined their anti-MM potential *in vitro* and *in vivo*.

2. Materials and methods

2.1. Cell culture

Human myeloma-derived cell line RPMI8226 secreting λ light chain, U266 secreting IgE λ light chain, NCI-H929 secreting IgA κ light chain, plasma cell leukemia (PCL) cell line ARH-77 secreting IgG κ light chain, human erythroleukemic cell line K562 and NK-92MI were introduced from the American Type Culture Collection (Manassas, VA). NK-92MI and transduced NK-92MI were incubated in alpha modification of Eagle's minimum essential medium (α-MEM; Invitrogen, Carlsbad, CA) supplemented with 2 mM L-glutamine, 0.2 mM inositol, 0.02 mM folic acid, 0.01 mM 2-mercaptoethanol, 12.5% FBS and 12.5% horse serum (Sigma–Aldrich Corporation, St Louis, MO). All the other cell lines were cultured in RPMI-1640 medium containing 10% FBS, 2 mM L-glutamine, penicillin (10 IU/ml) and streptomycin (100 μg/ml) (Invitrogen, Carlsbad, CA) at 37 °C in a humidified atmosphere with 5% CO2. Cells in the logarithmic growth phase were used for all experiments.

Peripheral blood samples collected from three healthy volunteers were processed by Ficoll–Paque density gradient centrifugation to obtain PBMNC. Primary myeloma cells were isolated from bone marrow aspirates of five newly diagnosed MM patients approved by the Institutional Ethics Committee (Shanghai Changzheng Hospital Institutional Ethics Committee). All participants provided their written informed consent. The patients with MM were hospitalized in Shanghai Changzheng Hospital from October 2009 to December 2012, including three males and two females with a median age of 53 years (ranging from 40 to 69 years) (Table 1). The mononuclear cells from the bone marrow aspirates were isolated using CD138 MACS MicroBeads and an automatic MACS magnetic cell sorter machine (Miltenyi, Bergisch Gladbach, Germany) according to the manufacturer's instructions and as described by Baumann et al. (2012). The primary myeloma cells were grown in the same medium as that for MM cell lines.